

Cartilage Tissue Engineering Using Dermis Isolated Adult Stem Cells: The Use of Hypoxia during Expansion versus Chondrogenic Differentiation.

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Public Summary:

Articular cartilage lacks an inherent ability to heal, and lesions often result in irreversible degeneration. Dermis isolated adult stem (DIAS) cells, a subpopulation of cells from the skin, is a promising source of cells for tissue engineering cartilage constructs. Hypoxia, or low oxygen, is known to have profound effects on mesenchymal stem cells. The objective of this study was to build upon the mechanistic knowledge of hypoxia and translate it to stimulating DIAS cells for cartilage tissue engineering, proliferate or produce cartilage-like matrix. DIAS cells were isolated and expanded in monolayer in hypoxic (5% oxygen) or normoxic (20% oxygen) conditions. Cells were then directed toward the chondrocyte lineage, or differentiated, for 2 weeks in small cell clusters, termed micromasses, on chondroitin sulfate, a matrix molecule found in cartilage. Monolayer cells were examined for proliferation rate and colony forming efficiency. Micromasses were assessed for cellular, biochemical, and histological properties. Differentiation in hypoxic conditions following normoxic expansion increased the per-cell basis of cartilage matrices, collagen type II and glycosaminoglycans (GAGs), by 2.3 fold and 1.2 fold, respectively, relative to continuous normoxic culture ($p < 0.0001$). Groups expanded in hypoxia produced 51% more collagen and 23% more GAGs than those expanded in normoxia ($p < 0.0001$). Hypoxia also limited cell proliferation in monolayer and in 3D culture. Collectively, these data show hypoxic differentiation following normoxic expansion significantly enhances chondrogenic differentiation of DIAS cells, improving the potential utility of these cells for cartilage engineering.

Scientific Abstract:

Dermis isolated adult stem (DIAS) cells, a subpopulation of dermis cells capable of chondrogenic differentiation in the presence of cartilage extracellular matrix, are a promising source of autologous cells for tissue engineering. Hypoxia, through known mechanisms, has profound effects on in vitro chondrogenesis of mesenchymal stem cells and could be used to improve the expansion and differentiation processes for DIAS cells. The objective of this study was to build upon the mechanistic knowledge of hypoxia and translate it to tissue engineering applications to enhance chondrogenic differentiation of DIAS cells through exposure to hypoxic conditions (5% O₂) during expansion and/or differentiation. DIAS cells were isolated and expanded in hypoxic (5% O₂) or normoxic (20% O₂) conditions, then differentiated for 2 weeks in micromass culture on chondroitin sulfate-coated surfaces in both environments. Monolayer cells were examined for proliferation rate and colony forming efficiency. Micromasses were assessed for cellular, biochemical, and histological properties. Differentiation in hypoxic conditions following normoxic expansion increased per cell production of collagen type II 2.3 fold and glycosaminoglycans 1.2 fold relative to continuous normoxic culture ($p < 0.0001$). Groups expanded in hypoxia produced 51% more collagen and 23% more GAGs than those expanded in normoxia ($p < 0.0001$). Hypoxia also limited cell proliferation in monolayer and in 3D culture. Collectively, these data show hypoxic differentiation following normoxic expansion significantly enhances chondrogenic differentiation of DIAS cells, improving the potential utility of these cells for cartilage engineering.